Cell Metabolism
Previews

Atherogenesis on the Chopping Block

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Endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) are now established features of the atherosclerotic plaque. In this issue, Thorp et al. (2009) provide initial insights into the causative relationship between UPR and the atherosclerotic disease process, specifically linking the proapoptotic mediator CHOP to plaque growth and necrosis.

Atherosclerosis is a progressive disease characterized by development of lipid-rich lesions in the subendothelial space of large arteries and the primary cause of heart disease and stroke. While the initial stages are relatively benign, increases in plaque size and complexity significantly elevate the risk of acute clinical events. The cellular and anatomical features of advanced human plaques that are unstable or so-called "vulnerable" to rupture include a large acellular necrotic core enriched in lipids and cell debris, a reduced smooth muscle cell-containing "fibrous cap," and an increased rate of inflammation and cell death (Virmani et al., 2006). The molecular mechanisms underlying these changes are of critical importance for clinical disease management. Whereas the actual causes underlying the cellular demise in lesions are not entirely understood, several cytotoxic molecules are known to be enriched in advanced atheromas (e.g., oxidized lipids, excessive amounts of free cholesterol, reactive oxygen radicals), and these can induce apoptotic cell death in macrophages, endothelial cells, and vascular smooth muscle cells (VSMC). A recent series of papers have demonstrated that ER stress and the UPR pathway is activated in human and mouse atherosclerotic lesions, implicating this pathway as a potential mechanism contributing to lesional cell death, inflammation, and disease progression (see Thorp et al., 2009). The in vivo experimental evidence for a role of UPR in plaque development, however, has been lacking. Thorp et al. now address this issue by specifically examining the deficiency of the proapoptotic UPR mediator CHOP in two common murine models of atherosclerosis (Thorp et al., 2009).

The UPR pathway activates cellular repair mechanisms, which are central to the ability of cell to cope with insults negatively affecting ER function. Under conditions of severe and irreversible damage, activation of the UPR can also lead to apoptosis, which is mediated by induction of transcription factor CHOP. While the mechanisms by which CHOP activates the apoptotic cell death are still being defined, its critical role in mediating the ER stress-induced apoptosis is well established (Mungrue et al., 2009; Oyadomari and Mori, 2004). Of particular significance to its proposed role in atherogenesis, CHOP expression is elevated in human atherosclerotic lesions, particularly in advanced plaques prone to rupture (Myoishi et al., 2007). In addition, in vitro studies in macrophages and vascular smooth muscle cells have shown that CHOP deficiency or siRNA-mediated knockdown of CHOP protects cells from apoptosis induced by molecules enriched in atheromas, such as free cholesterol loading or treatment with 7-ketocholesterol (Feng et al., 2003; Myoishi et al., 2007).

To interrogate the impact of CHOP deficiency on atherosclerotic plaque formation, Thorp et al. placed Chop$^{-/-}$ mice onto Apoe$^{-/-}$ and Ldlr$^{-/-}$ background, two common murine models of atherogenesis. They show that Chop$^{-/-}$/Apoe$^{-/-}$ double-knockout mice develop significantly less lesions in aortic root (~35%) than their wild-type CHOP counterparts when fed a high-fat and high-cholesterol diet for 10 weeks. To confirm these findings, the authors also carried out a second study, this time utilizing the Chop$^{-/-}$/Ldlr$^{-/-}$ model and obtained essentially the same results. Interestingly, no differences could be observed at early stages of lesion development (4 weeks on diet). Even though only relatively small number of animals were utilized (n = 6 per group), these data suggest that CHOP affects lesion formation primarily in the more advanced stages. As CHOP deficiency is predicted to reduce apoptosis in the context of ER stress, Thorp et al. (2009) went on to characterize the lesions for features of apoptotic cell death and plaque necrosis. In both animal models, necrotic areas present in atheromas of Chop$^{-/-}$ mice were substantially reduced. Importantly, this decrease was more robust (~40%–50%) than one would expect to occur solely based on a decrease in total plaque area (35%). They postulate that macrophage apoptosis represents a major contributing factor to necrotic core enlargement. To support these claims they analyzed a subgroup of mice from Apoe$^{-/-}$ cohort with identical mean lesion size and demonstrate reduced necrosis in this Chop$^{-/-}$/Apoe$^{-/-}$ subgroup. Although similar analysis was not carried out in Ldlr$^{-/-}$, these data do support a role of CHOP as a contributing factor in intimal apoptotic cell death and necrotic core formation. Indeed, lesions from Chop$^{-/-}$ mice in both models contained a lower number of cells exhibiting signs of apoptosis. Given that macrophages represent the major cell type occupying the intima and surround the necrotic core, the authors suggest that macrophage apoptosis is an important contributor to observed changes in plaque necrosis. They support this hypothesis by in situ immunohistochemistry analyses of apoptotic cell distributions. Thorp et al. also demonstrate that Chop$^{-/-}$ peritoneal macrophages are resistant to apoptosis induced by several relevant ER stressors and that CHOP deficiency has no apparent

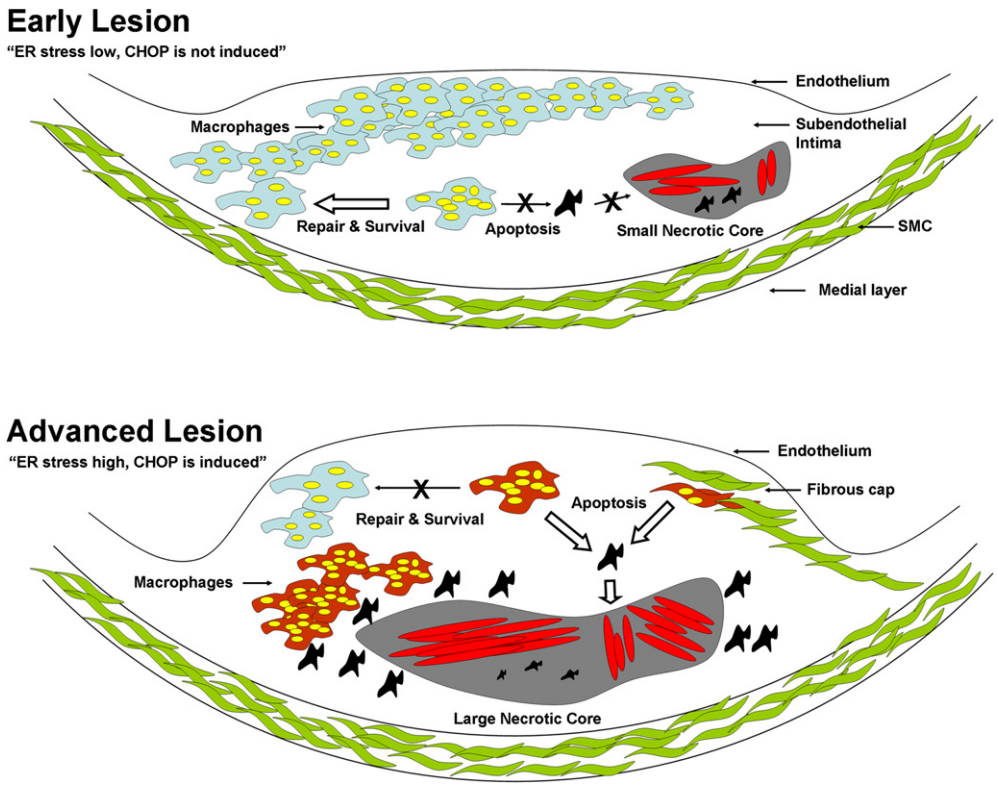

Figure 1. Schematic Diagram Illustrating Potential Roles of ER Stress and CHOP in the Atherosclerotic Lesion Development
Early lesions are exemplified by so called "fatty streaks" containing cholesterol-enriched "foamy" macrophages (shown in blue, with yellow lipid droplets) in the subendothelial space of lesion intima. During these early stages of the lesion development, the level of cytotoxic substances (e.g., oxysterols) is insufficient to overwhelm the metabolic capacity of macrophages, and the CHOP branch of the UPR is not activated. Under these conditions, the UPR activation leads primarily to cell repair, very low occurrence of apoptosis (shown in black), and minimal or absent necrotic core (shown in gray with red cholesterol crystals and black cell debris). As the lesion progresses to its advanced stages, the level of ER stress in macrophages becomes too severe (indicated as orange-colored macrophages), leading to CHOP induction and apoptosis. The apoptotic cells, if not cleared via phagocytosis, can undergo secondary necrosis and amplification of the necrotic core size. It is plausible that similar processes are also taking place in other cell types, such as smooth muscle cells (shown in green), resulting in thinning of the fibrous cap layer and further destabilization of the atherosclerotic plaque.

effect on the ability of macrophages to phagocytose apoptotic cells in vitro. On the contrary, the ability of macrophages to clear apoptotic cells can be compromised in atherosclerotic plaques (Schrijvers et al., 2005). The actual impact of CHOP deficiency on phagocytic function of macrophages has not been evaluated in vivo, and therefore it cannot be completely excluded.

The current study by Thorp et al. adds important relevance to previous observations illustrating activation of UPR pathway in atherosclerotic lesions. As UPR induction can lead to either cell repair or apoptosis, it was not clear to which extent this pathway truly contributes to lesional cell death and atherogenesis in vivo. This study provides the first direct causal link between UPR and atherosclerotic disease and strongly supports the hypothesis that ER stress, via CHOP-mediated apoptosis, enhances advanced lesion development and the occurrence of plaque necrosis (see Figure 1). The net contribution of macrophages to these findings is strongly implied, but as authors point out, further studies are needed to address this issue. As a case in point, apoptosis of VSMCs can also enhance atherosclerosis, increase the size of the necrotic core, and lead to thinning of the fibrous cap (Clarke et al., 2008). CHOP is known to be ubiquitously expressed, and the siRNA-mediated knockdown of CHOP in human VSMCs reduces the apoptotic cell death in response to 7-ketocholesterol (Myoishi et al., 2007). Furthermore, the UPR molecules in endothelial cells were also shown to be elevated in human atherosclerosis (Gargalovic et al., 2006). Therefore, it remains to be resolved whether CHOP deficiency also affects apoptosis of smooth muscle or endothelial cells in lesions as well as other important features of the vulnerable plaque (e.g., fibrous cap thickness). Nevertheless, this work provides an important piece of the puzzle to the proposed role of UPR in atherogenesis and highlights yet another example of disease where mitigating the CHOP function may be of therapeutic benefit (Oyadomari et al., 2002; Tamaki et al., 2008).

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Do Cancer Cells Care If Their Host Is Hungry?

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A recent report by Kalaany and Sabatini concerning mechanisms underlying the inhibitory effect of dietary restriction on the growth of certain tumors adds to the evidence that insulin and IGF-I are hormones with relevance to oncology.

Recent progress in the field of "cancer energetics" involves descriptions of the influence of oncogenes and tumor suppressor genes on the metabolic pathways used by cancer cells to generate ATP (Jones and Thompson, 2009). However, an important gap in knowledge in this field involves an issue beyond the cellular level—the influence of whole organism energy balance on cancer biology. The strong inhibitory effect of host dietary restriction on the growth of certain experimental tumors is a classic observation that predates even Warburg's work concerning cancer energetics, yet studies concerning the mechanisms underlying this phenomenon are sparse.

A recent paper (Kalaany and Sabatini, 2009) provides important data which add to prior evidence that the inhibitory effect of dietary restriction on tumor growth is attributable to the effect of the diet on insulin and insulin-like growth factors (Figure 1). They confirm that restriction of food intake lowers both insulin and IGF-I levels and show that the degree of in vitro mitogenic responsivity of various cell lines to insulin or IGF-I can be used to predict which corresponding xenografts will be growth inhibited in vivo by dietary restriction. Furthermore, they demonstrate that PI3 kinase-activating mutation or loss of function of PTEN is sufficient to confer resistance to the growth inhibitory effects of dietary restriction.

A simple model to account for these observations is that some cancers are responsive to insulin and/or IGFs, and that these neoplasms thrive when levels of these mitogens are sufficient to contribute to activation of the PI3K pathway. Many studies are consistent with this hypothesis. The implicated receptors are expressed by many human cancers (e.g., Law et al., 2008), and epidemiologic data (reviewed in Pollak, 2008) provide evidence that high circulating levels of the implicated ligands are associated with adverse cancer prognosis and/or increased cancer risk. Examples of consistent experimental data include findings that tumor growth is reduced in mice with mutations that lower IGF-I levels (Majeed et al., 2005), and that the growth inhibitory effect of caloric restriction on a bladder cancer model can be abolished by infusing IGF-I (Dunn et al., 1997).

However, the simplest model may not be complete. The dietary restriction employed by Kalaany and Sabatini would be expected to result in alterations in additional hormones (such as leptin, adiponectin, FGF21, and/or glucagon), and an influence of these on neoplasms has not been excluded. Additional work to compare in vitro the influences of physiologic concentrations of these hormones to those of insulin and IGF-I would be of interest. Measurements at later time points of in vivo changes in AKT pathway activation within cancers as a function of host diet would also be relevant, as some data reported concern tumors less than 20 mm^3.

Apart from the effects of dietary restriction on the hormonal environment of cancers, does it reduce energy supply to the tumor? Kalaany and Sabatini do not report changes in glucose, amino acids, or fatty acids associated with the degree of caloric restriction employed. Such measurements would be of interest, but it is likely that the host breaks down muscle and fat at sufficient rates to maintain circulating energy supply to normal tissues, and to cancers. Therefore, host dietary restriction may inhibit growth of certain cancers through changes in hormone concentrations rather than by causing energy depletion at the cellular level.

What about the role of the energy sensor AMP-activated kinase in tumor growth inhibition by dietary restriction? In single-cell organisms and in studies of cancer cells in vitro, reduced supply of energy sources leads to activation of AMPK, which